

## Secondary Metabolites from *Asphodelus aestivus*

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Z. Naturforsch. **61b**, 1304–1310 (2006); received March 21, 2006

Together with ten well known compounds, the quinic acid derivative chlorogenic acid, the nucleoside adenosine, two amino acids, tryptophan and phenylalanine, the anthraquinone derivatives, aloemodin, aloemodin acetate and chrysothanol 1-*O*-gentiobioside, the flavone C-glycosides, isovitexin, isoorientin and isoorientin 4'-*O*- $\beta$ -glucopyranoside, as well as two new acylated isoorientin derivatives, 6''-*O*-(malonyl)-isoorientin and 6''-*O*-[(*S*)-3-hydroxy-3-methylglutaroyl]-isoorientin, were isolated from the water soluble part of the methanolic extract of the fresh leaves of *Asphodelus aestivus*. All compounds were structurally identified by spectroscopic methods, including UV, MS, and NMR (1D and 2D) spectroscopy. Among the compounds isolated, chlorogenic acid and isoorientin were found to be the main compounds of the methanolic extract.

**Key words:** *Asphodelus aestivus*, Liliaceae, Secondary Metabolites, Acylated Flavone C-Glycosides

### Introduction

*Asphodelus aestivus* Brot. is one of the three *Asphodelus* L. species represented in the flora of Turkey [1]. A severe neurologic syndrome accompanied by intense neuronal pigmentation in sheep in Turkey was observed after ingestion of *A. aestivus*. The disease was characterized by neurological signs, such as paresis, ataxia and convulsions and the pathological findings were recently reported [2]. Previous phytochemical investigations performed on *Asphodelus* species resulted in the isolation of anthranoides, flavonoids and triterpenes [3–6]. However, all compounds have been isolated from the tubers of *Asphodelus* species and not from the ingested aerial parts. These observations prompted us to investigate for the secondary metabolites of the fresh leaves of *A. aestivus* with the aim to isolate important representatives for further biological *in vitro* and *in vivo* testing. Thus, we report in the present study on the isolation of two new flavone C-glycosides, 6''-*O*-(malonyl)-isoorientin (**11**) and 6''-*O*-[(*S*)-3-hydroxy-3-methylglutaroyl]-isoorientin (**12**). Besides ten known compounds, namely three anthranoides (1,8-dihydroxy-anthraquinones), three known

flavone C-glycosides, one nucleoside, two amino acids and a quinic acid derivative were isolated.

### Experimental Section

#### General experimental procedures

UV spectra were recorded on a HP Agilent 8453 spectrophotometer. Optical rotations were measured on a Rudolph Autopol IV polarimeter. 1D and 2D NMR spectra were recorded on Bruker AMX (Avance) 300, 400 and 600 MHz spectrometers at 295 K and referenced against the respective residual non deuterated solvent (see Tables 1 and 2). ESIMS spectra were measured on a ThermoQuest Finnigan 7000 with a spray voltage of 4 kV. HR-LSIMS were recorded on a Finnigan MAT 95 (Cs<sup>+</sup>, 15 kV). TLC analyses were carried out on silica gel 60 F<sub>254</sub> precoated plates (0.2 mm; Merck, Darmstadt). 1% Vanillin/H<sub>2</sub>SO<sub>4</sub> was used as detection reagent. For medium-pressure liquid chromatographic (MPLC) separations, a Büchi B pump, a LKB 17000 Minirac fraction collector, a Rheodyne injector, and Büchi columns (column dimensions 2.6 × 46 cm, and 1.8 × 35 cm) were used. Silica gel 60 (0.063–0.200 mm; Merck, Darmstadt), Polyamide (Fluka) and Sephadex LH-20 (Fluka) were utilized for open column chromatography (CC). LiChroprep RP-18 (25–40  $\mu$ m, Merck) material was used for MPLC.

Table 1. The <sup>1</sup>H NMR spectroscopic data for flavon C-glycosides **8**–**12** (δ ppm, m, *J* in Hz).

H Atom	<b>8</b> 300 MHz, CD <sub>3</sub> OD	<b>9</b> 300 MHz, DMSO	<b>10</b> 600 MHz, CD <sub>3</sub> OD	<b>11</b> 400 MHz, CD <sub>3</sub> OD	<b>11</b> 600 MHz, DMSO	<b>12</b> 500 MHz, DMSO
<i>Flavon</i>						
3	6.60 s	6.66 s	6.63 s	6.51 s	6.67 s	6.66 s
8	6.50 s	6.48 s	6.51 s	6.46 s	6.48 s	6.46 s
2'	7.92 d (8.8)	7.39 d (2.2)	7.22 d (8.5)	7.34 d (2.2)	7.40 d (2.2)	7.40 d (2.2)
3'	7.85 d (8.8)	–	–	–	–	–
5'	7.85 d (8.8)	6.89 d (8.3)	7.32 d (8.5)	6.88 (8.4)	6.89 d (8.3)	6.89 d (8.3)
6'	7.92 d (8.8)	7.41 dd (8.3, 2.2)	7.45 dd (8.5, 2.0)	7.35 dd (8.4, 2.2)	7.42 dd (8.3, 2.2)	7.41 dd (8.3, 2.2)
5-OH	–	13.57 s	–	–	13.58 s	13.56 s
<i>6-C-Glucosyl</i>						
1''	4.90 d (9.4)	4.58 d (9.8)	4.88 d (9.9)	4.88 d (9.9)	4.59 d (9.8)	4.62 d
2''	4.16 t (9.4)	4.03 dd (9.8, 9.2)	4.16 t (9.9)	4.18 dd (9.9, 9.0)	4.08 dd (9.8, 9.0)	4.10 t
3''	3.51 <sup>a</sup>	3.22–3.11 <sup>a</sup>	3.50 <sup>a</sup>	3.48 <sup>a</sup>	3.22 t (9.0)	3.22 <sup>a</sup>
4''	3.47 <sup>a</sup>	3.22–3.11 <sup>a</sup>	3.49 <sup>a</sup>	3.47 <sup>a</sup>	3.18 t (9.0)	3.18 <sup>a</sup>
5''	3.42 m	3.22–3.11 <sup>a</sup>	3.42 <sup>a</sup>	3.62 m	3.40 <sup>a</sup>	3.38 <sup>a</sup>
6''	3.88 dd (12.2, 2.2)	3.68 dd (11.8, 1.5)	3.87 dd (12.0, 2.0)	4.51 dd (12.0, 1.8)	4.43 br d (12.0)	4.36 br d (12.0)
	3.74 dd (12.2, 5.3)	3.40 dd (11.8, 6.0)	3.74 dd (12.0, 5.4)	4.30 dd (12.0, 5.8)	3.96 dd (12.0, 7.0)	3.93 dd (12.0, 7.0)
<i>4'-O-Glucosyl</i>						
1'''			4.94 d (7.5)			
2'''			3.55 dd (7.5, 8.6)			
3'''			3.51 <sup>a</sup>			
4'''			3.43 <sup>a</sup>			
5'''			3.48 <sup>a</sup>			
6'''			3.92 dd (12.0, 2.0)			
			3.71 dd (12.0, 5.4)			
<i>6''-O-Malonyl</i>						
CH <sub>2</sub>				missing	3.31 <sup>a</sup>	
<i>6''-O-HMGlutaryl</i>						
2'''						2.62 d (13.8), 2.51 d (13.8)
4'''						2.50 d (14.9), 2.40 d (14.9)
<i>CH<sub>3</sub></i>						
						1.30 s

<sup>a</sup> Unclear signal pattern due to overlapping.

#### Plant material

*Asphodelus aestivus* Brot. (Liliaceae) was collected from Kusadasi, Aydın, W. Anatolia, in Turkey at August 20, 2002. The plant material was identified by Dr. Bengi Erdağ (Department of Biology, Faculty of Arts and Sciences, University of Adnan Menderes, Aydın, Turkey). The voucher specimen (AYDN T-29) has been deposited at the Herbarium of the AYDN, Faculty of Science & Arts, Adnan Menderes University, Aydın, Turkey.

#### Extraction and isolation

The fresh leaves of *A. aestivus* (650 g) were cut into small pieces and macerated with MeOH (5 l) at r. t. for three days. The methanolic extract was evaporated to dryness *in vacuo*. The crude extract was suspended in H<sub>2</sub>O and partitioned against CH<sub>2</sub>Cl<sub>2</sub>. The lyophilised H<sub>2</sub>O extract (28.3 g) was divided into two equal parts and each was subjected to C<sub>18</sub>-Medium Pressure Liquid Chromatography (C<sub>18</sub>-MPLC, column dimension: 2.6 × 46 cm) with H<sub>2</sub>O, 25% MeOH,

50% MeOH and MeOH as eluents to obtain four main fractions, A–D. Similar fractions obtained from the two columns were combined after TLC control. Fraction B (1200 mg) was likewise applied to C<sub>18</sub>-MPLC (column dimensions: 2.6 × 46 cm) using a MeOH gradient (0–50%) to obtain **1** (chlorogenic acid, 350 mg) as well as two subfractions, B<sub>1–2</sub>. Purification of subfraction B<sub>1</sub> (52 mg) by CC on SiO<sub>2</sub> gel (EtOAc-MeOH-H<sub>2</sub>O, 100 : 10 : 5) furnished **3** (tryptophan, 5 mg) and **4** (phenylalanine, 9 mg). Repeated CC of subfraction B<sub>2</sub> (38 mg) on a SiO<sub>2</sub> gel (EtOAc-MeOH-H<sub>2</sub>O, 100 : 10 : 5) gave **2** (adenosine, 16 mg). Fraction C (1030 mg) was subjected to C<sub>18</sub>-MPLC (column dimensions: 2.4 × 32 cm), and eluted with MeOH-H<sub>2</sub>O mixtures (25–100% MeOH) to yield seven fractions, C<sub>1–7</sub>. Repeated CC of fraction C<sub>2</sub> (94 mg) on SiO<sub>2</sub> gel (CH<sub>2</sub>Cl<sub>2</sub>-MeOH-H<sub>2</sub>O, 80 : 20 : 2 to 60 : 40 : 10) and Sephadex LH-20 (MeOH), respectively gave **10** (isoorientin 4'-*O*-glucopyranoside, 4 mg). Fraction C<sub>4</sub> (118 mg) was rechromatographed on a SiO<sub>2</sub> column (EtOAc-MeOH-H<sub>2</sub>O, 100 : 17 : 13) to afford **9** (isoorientin, 62 mg). Compounds **12** {6''-*O*-[(*S*)-3-hydroxy-3-

Table 2. The  $^{13}\text{C}$  NMR spectroscopic data for flavon C-glycosides, **8**–**12** ( $\delta$  ppm).

C Atom	<b>8</b> MeOD	<b>9</b> DMSO-d <sub>6</sub>	<b>10</b> MeOD	<b>11</b> MeOD	<b>11</b> DMSO-d <sub>6</sub>	<b>12</b> DMSO-d <sub>6</sub>
<i>Flavon</i>						
	Apigenin	Luteolin	Luteolin	Luteolin	Luteolin	Luteolin
2	166.4	163.6	165.4	166.2	163.6	163.8
3	104.1	102.8	105.3	104.0	102.7	102.8
4	184.2	181.9	184.0	184.0	181.8	181.4
5	163.0	160.7	162.1	162.1	160.5	161.2
6	109.4	108.9	109.5	108.8	108.4	108.5
7	165.2	163.3	165.0	164.9	163.1	163.6
8	95.4	93.5	95.4	95.2	93.4	93.4
9	158.9	156.2	158.7	158.8	156.1	156.2
10	105.3	103.4	105.4	105.2	103.3	103.3
1'	123.3	121.4	127.1	123.6	121.3	121.4
2'	129.6	113.3	115.0	114.2	113.2	113.3
3'	117.2	145.7	148.7	147.0	145.6	145.7
4'	162.2	149.7	150.1	151.0	149.6	149.7
5'	117.2	116.0	118.0	116.8	116.0	116.0
6'	129.6	119.0	119.9	120.3	118.9	118.9
<b>5-OH</b>						
<i>6-Glucosyl</i>						
1''	75.5	73.1	75.3	75.4	72.9	73.1
2''	72.8	70.6	72.6	72.4	69.8	69.9
3''	82.8	81.6	82.7	79.9	78.5	78.7
4''	72.0	70.2	71.9	71.8	70.3	70.5
5''	80.3	79.0	80.2	79.7	78.0	78.1
6''	63.0	61.5	62.9	65.9	65.2	64.1
<i>4'-O-Glucosyl</i>						
1'''			103.2			
2'''			74.8			
3'''			78.6			
4'''			71.3			
5'''			77.6			
6'''			62.5			
<i>6''-O-Malonyl</i>						
CH <sub>2</sub>				missing <sup>a</sup>	41.5	
RCOOR'				168.8	167.9	
RCOOH				170.8	167.0	
<i>6''-O-HMGlutaroyl</i>						
1''' (RCOOR')						170.5
2'''						45.3
3'''						69.0
4'''						45.4
5''' (RCOOH)						172.6
Me						27.3

<sup>a</sup> The signal is missing due to overlapping by solvent signal.

methylglutaroyl]-isorientin, 10 mg} and **8** (isovitexin, 14 mg) were purified from fraction C<sub>6</sub> (104 mg) by CC using Sephadex LH-20 (MeOH) and SiO<sub>2</sub> gel (EtOAc-MeOH-H<sub>2</sub>O, 100:15:0 to 100:17:13) as stationary phase. Fraction D (152 mg) was applied to CC on SiO<sub>2</sub> gel using a gradient of CH<sub>2</sub>Cl<sub>2</sub>-MeOH-H<sub>2</sub>O (80:20:1 to 50:50:5) to give **7** (chrysophanol 1-*O*-gentiobioside, 8 mg).

In order to isolate the phenolic compounds, which were present in small amounts in the previous extract, we increased the amount of the material (2 kg) and extracted with MeOH (8 l). The methanolic extract was evaporated to dry-

ness *in vacuo*. The crude extract was suspended in H<sub>2</sub>O and partitioned against CH<sub>2</sub>Cl<sub>2</sub>. The lyophilised H<sub>2</sub>O extract (75 g) was mounted on a column with polyamide as stationary phase. Elution with H<sub>2</sub>O and the increasing amounts of MeOH in H<sub>2</sub>O (10–100%) yielded nine main fractions, A–I. Fraction G (2.317 g) was dissolved in H<sub>2</sub>O and extracted with EtOAc. The lyophilised EtOAc extract (1 g) was applied to CC on SiO<sub>2</sub> gel using mixtures of EtOAc-MeOH-H<sub>2</sub>O (100:10:2 to 100:20:15) as mobile phase to give four subfractions, G<sub>1–4</sub>. Subfraction G<sub>1</sub> (400 mg) was fractionated by CC over SiO<sub>2</sub> gel to obtain two subfractions,

G<sub>1a–b</sub>. Compounds **6** (aloe-emodin acetate, 5 mg) and **5** (aloe-emodin, 7 mg) were purified by using the same method (CC on Sephadex LH-20, MeOH) from fractions G<sub>1a</sub> (90 mg) and G<sub>1b</sub> (80 mg) respectively. Fraction I (250 mg) was applied to a polyamide column utilising CH<sub>2</sub>Cl<sub>2</sub>-MeOH-acetone-ethylmethylketone (3 : 2 : 1 : 1) to give two subfractions, I<sub>1–2</sub>. 6''-O-(Malonyl)-isoorientin (**11**, 15 mg) was purified from subfraction I<sub>1</sub> (114 mg) by CC on Sephadex LH-20 (cyclohexane-aceton-CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 1 : 1 : 1 : 2).

#### Isovitexin (**8**)

Amorphous yellow powder. – UV/vis (MeOH):  $\lambda_{\max}$  = 270, 302 and 335 nm. – <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): Table 1. – <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD): Table 2. – MS (ESI):  $m/z$  = 433 [M+H]<sup>+</sup> and 431 [M-H]<sup>-</sup>, calcd. for C<sub>21</sub>H<sub>20</sub>O<sub>10</sub>.

#### Isoorientin (**9**)

Amorphous yellow powder. – UV/vis (MeOH):  $\lambda_{\max}$  = 255, 270 and 350 nm. – <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): Table 1. – <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): Table 2. – MS (ESI):  $m/z$  = 449 [M+H]<sup>+</sup> and 447 [M-H]<sup>-</sup>, calcd. for C<sub>21</sub>H<sub>20</sub>O<sub>11</sub>.

#### Isoorientin 4'-O-glucopyranoside (**10**)

Amorphous yellow powder. – UV/vis (MeOH):  $\lambda_{\max}$  = 240, 273 and 335 nm. – <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD): Table 1. – <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD): Table 2. – MS (ESI):  $m/z$  = 611 [M+H]<sup>+</sup>, 633 [M+Na]<sup>+</sup>, 1243 [2M+Na]<sup>+</sup> and 609 [M-H]<sup>-</sup>, 1219 [2M-H]<sup>-</sup>, calcd. for C<sub>27</sub>H<sub>30</sub>O<sub>16</sub>.

#### 6''-O-(Malonyl)-isoorientin (**11**)

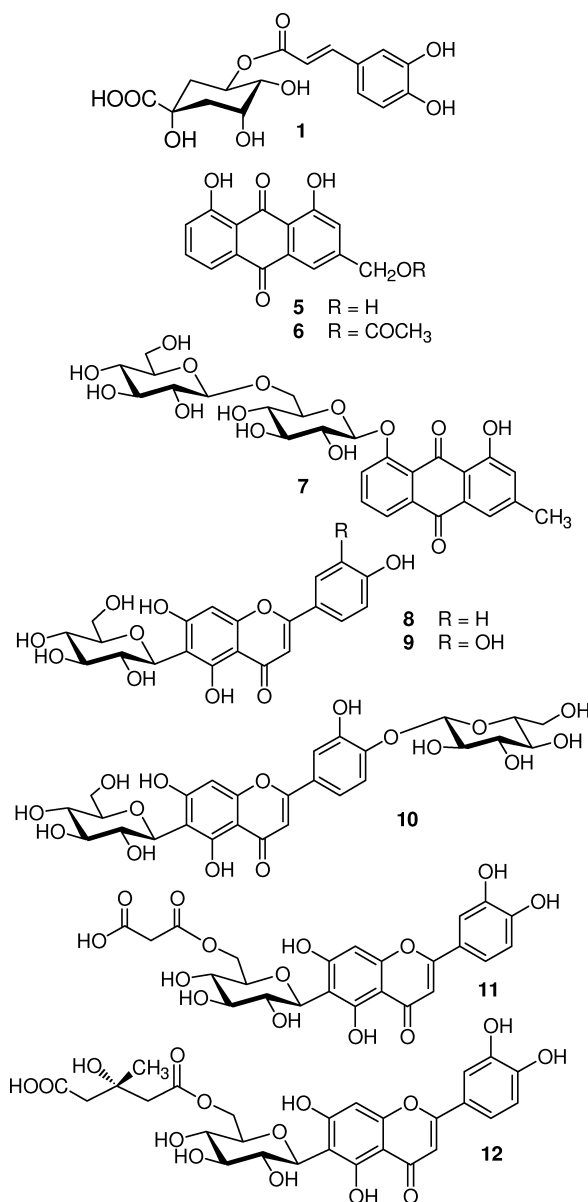
Amorphous yellow powder. –  $[\alpha]_D^{25} + 10.9$  (c 0.1, MeOH). – UV/vis (MeOH):  $\lambda_{\max}$  = 211, 255(sh), 271, 348 nm. – <sup>1</sup>H NMR (600 MHz, DMSO-d<sub>6</sub>): Table 1. – <sup>13</sup>C NMR (150 MHz, DMSO-d<sub>6</sub>): Table 2. – MS (HR-LSI):  $m/z$  found 535.1087 [M+H]<sup>+</sup>, calcd. 535.1088 (C<sub>24</sub>H<sub>22</sub>O<sub>14</sub>). – MS (ESI):  $m/z$  = 535 [M+H]<sup>+</sup> and 533 [M-H]<sup>-</sup>.

#### 6''-O-[(S)-3-hydroxy-3-methylglutaroyl]-isoorientin (**12**)

Amorphous yellow powder. –  $[\alpha]_D^{25} + 2.9$  (c 0.1, MeOH). – UV/vis (MeOH):  $\lambda_{\max}$  = 210, 255(sh), 271, 340 nm. – <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>): Table 1. – <sup>13</sup>C NMR (125 MHz, DMSO-d<sub>6</sub>): Table 2. – MS (HR-LSI):  $m/z$  found 593.1497 [M+H]<sup>+</sup>, calcd. 593.1506 (C<sub>27</sub>H<sub>28</sub>O<sub>15</sub>). – MS (ESI):  $m/z$  = 593 [M+H]<sup>+</sup> and 591 [M-H]<sup>-</sup>.

## Results and Discussion

Chromatographic studies on the water soluble part of the methanolic extract of fresh leaves of *As-*



*phodelus aestivus* yielded compounds **1–12**, chlorogenic acid (**1**), adenosine (**2**), tryptophan (**3**), phenylalanine (**4**), aloe-emodin (**5**), aloe-emodin acetate (**6**), chrysophanol 1-O-gentiobioside (**7**), and five flavon C-glycosides, isovitexin (**8**), isoorientin (**9**) and isoorientin 4'-O- $\beta$ -glucopyranoside (**10**), 6''-O-(malonyl)-isoorientin (**11**) and 6''-O-[(S)-3-hydroxy-3-methylglutaroyl]-isoorientin (**12**).

The main components of the extract were a series of  $\beta$ -D-glucopyranosides of apigenin and luteolin show-

ing a C-C linkage to C-6 of the flavon nucleus (**8–12**). All of them were obtained as yellow amorphous solids and the UV data supported their flavonoid structure. The  $^1\text{H}$  NMR spectra of compounds **8–12** showed two singlets corresponding to the protons of H-3 and H-8 in rings C and A of a flavone, respectively (Table 1). All  $^{13}\text{C}$  NMR spectra exhibited a signal between  $\delta = 73.0$  and  $76.0$  characteristic to an anomeric carbon (C-1'') of a glucose moiety C-C connected to the aglycone (Table 2). The site of the C-glycosidation position on the ring A in **8–12** was established to be at C-6 of the flavone moiety by the appearance of long-range  $^1\text{H}$ ,  $^{13}\text{C}$ -correlations between the glucosyl anomeric proton (H-1'') and the carbon signals assigned as C-5 ( $\sim \delta = 161.0$ ), C-6 ( $\sim \delta = 108.5$ ) and C-7 ( $\sim \delta = 163.5$ ) in the  $^1\text{H}$ ,  $^{13}\text{C}$  HMBC experiments. The NMR signals of the sugar units and the flavonoids were completely assigned from  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^1\text{H}$ ,  $^1\text{H}$  COSY,  $^1\text{H}$ ,  $^{13}\text{C}$  HSQC and  $^1\text{H}$ ,  $^{13}\text{C}$  HMBC experiments.

In addition to common observations mentioned above, the  $^1\text{H}$  NMR spectrum of compound **8** (Table 1) showed additional signals of two sets of doublets as an AA'BB' system at  $\delta = 7.92$  and  $7.85$  (each 2H, d,  $J = 8.8$  Hz), due to the protons of H-2'/H-6' and H-3'/H-5' of ring B, respectively. These clearly supported the presence of an apigenin type flavone moiety for **8**. The  $^{13}\text{C}$  NMR resonances (Table 2) assigned for **8** were also in good agreement to literature values [7, 8]. Based on this observations, the structure of **8** was established as isovitexin.

In the  $^1\text{H}$  NMR spectrum of compound **9**, the signals of ring B resonated as an ABX system at  $\delta = 7.39$  (1H, d,  $J = 2.2$  Hz, H-2'),  $6.89$  (1H, d,  $J = 8.3$  Hz, H-5'), and  $7.41$  (1H, dd,  $J = 8.3$  and  $2.2$  Hz, H-6') indicating the presence of a 1,3,4-trisubstitution on ring B as in luteolin. The  $^{13}\text{C}$  NMR resonances (Table 2) of **9** were in good agreement with those reported for isoorientin [7, 8]. The molecular formula of **9** was determined as  $\text{C}_{21}\text{H}_{20}\text{O}_{11}$  by NMR data (Tables 1 and 2) and ESIMS which showed *pseudomolecular* ion peaks at  $m/z = 449$   $[\text{M}+\text{H}]^+$  and  $447$   $[\text{M}-\text{H}]^-$  confirming the proposed structure.

The ESIMS spectra of compound **10** showed quasi-molecular ion peaks at  $m/z = 611$   $[\text{M}+\text{H}]^+$ ,  $633$   $[\text{M}+\text{Na}]^+$  and  $1243$   $[2\text{M}+\text{Na}]^+$  in the positive and  $609$   $[\text{M}-\text{H}]^-$  in the negative mode and provided together with the  $^{13}\text{C}$  NMR data a molecular formula of  $\text{C}_{27}\text{H}_{30}\text{O}_{16}$ . Its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra exhibited a second anomeric proton signal and a corresponding

carbon resonance at  $\delta_{\text{H}} = 4.94$  (d,  $J = 7.5$  Hz, H-1'') and  $\delta_{\text{C}} = 103.2$  (CH, C-1''), respectively. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR resonances (Tables 1 and 2) assigned to the second sugar unit indicated the presence of an additional O-linked glucose. Based on the coupling constant of the anomeric proton ( $J = 7.5$  Hz), its configuration was found to be  $\beta$ . The chemical shifts of the ABX system attributed to the ring B were differing from those of isoorientin (**9**) and its derivatives (**11–12**) which have no glucosidation on ring B. A downfield shift of  $\sim 0.4$  ppm observed for H-5' ( $\delta = 7.32$ , d,  $J = 8.3$  Hz) indicated the hydroxy group at C-4' as glycosidation site of the second glucose moiety. This assumption was verified by a HMBC experiment showing the long-range correlation between the anomeric proton ( $\delta = 4.94$ ) of the glucose and C-4' ( $\delta = 150.1$ ). Based on these results, the structure of **10** was established as isoorientin 4'-O- $\beta$ -glucopyranoside [9–11].

HR-LSIMS of **11** showed a *pseudomolecular* ion  $[\text{M}+\text{H}]^+$  at  $m/z = 535.1087$ , which corresponded to the molecular formula  $\text{C}_{24}\text{H}_{22}\text{O}_{14}$ . The low resolution ESIMS of **11** showed *pseudomolecular* ion peaks at  $m/z = 535$   $[\text{M}+\text{H}]^+$  in the positive and  $533$   $[\text{M}-\text{H}]^-$  in the negative ion mode. The molecular weight of **11** was 86 mass units larger compared to that of isoorientin (**9**) suggesting a substitution with a malonyl group, which was also inferred from the fragment ions at  $m/z = 449$   $[\text{M}-\text{malonyl}+\text{H}]^+$  and  $447$   $[\text{M}-\text{malonyl}-\text{H}]^-$  characteristic of a malonic acid ester [12].

In the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **11** measured most signals showed signal pattern and shift values matching for isoorientin (Tables 1 and 2). Significant differences were observed for the hydroxymethylene protons (H-6''a and H-6''b) of the glucose unit showing a downfield shift of  $\sim 0.7$  and  $\sim 0.45$  ppm in the  $^1\text{H}$  NMR spectrum due to acylation ( $\delta = 4.43$  and  $3.96$ , respectively). Acylation effects were also observed for the C-6'' and C-5'' carbon resonances of the glucose moiety, which were shifted downfield ( $+3.7$  ppm) and upfield ( $-1.0$  ppm) due to  $\alpha$ - and  $\beta$ -effect of acylation, respectively. Two carbonyl carbon signals of a malonyl unit were observed at  $\delta = 167.0$  and  $167.9$ . The site of acylation was also supported by a long-range correlation between the glucose H-6''a, H-6''b and the carbonyl carbon of the malonyl moiety in the HMBC experiment. The proton and carbon resonances of the methylene functionality of the malonyl unit were observed at  $\delta_{\text{H}} = 3.31$  and  $\delta_{\text{C}} = 41.5$  and showed correlation to each other in HSQC experiment confirm-

ing the presence of the malonyl moiety in **11**. The use of MeOD instead of DMSO- $d_6$  as NMR solvent is less suitable, because the proton and carbon resonances for the methylene of the malonyl group can not be observed due to an overlap with the solvent signal (Tables 1 and 2). Similar observations were also reported for malonylated flavonol glycosides measured in MeOD [13]. Consequently, the structure of compound **11** was established as the previously not known 6''-*O*-(malonyl)-isoorientin.

HR-LSIMS of **12** showed a *pseudomolecular* ion  $[M+H]^+$  at  $m/z = 593.1497$  corresponding to a molecular formula of  $C_{27}H_{28}O_{15}$ . Accordingly, the LR-ESIMS of **12** showed *pseudomolecular* ion peaks at  $m/z = 593 [M+H]^+$  in the positive and  $591 [M-H]^-$  in the negative ion mode. The  $^1H$  NMR spectrum of **12** exhibited similar proton resonances for the luteolin moiety as in isoorientin (**9**) (Table 1). Additionally, two methylene protons resonating as AB systems ( $\delta = 2.62$  and  $2.51$ ,  $J_{AB} = 13.8$  Hz;  $2.50$  and  $2.40$ ,  $J_{AB} = 14.9$  Hz) and a methyl resonance (3H,  $\delta = 1.30$  s) were observed. Moreover, in the  $^{13}C$  NMR spectrum (Table 2), six additional carbon signals were present: two carbonyls ( $\delta = 172.6$  and  $170.5$ ), an oxygenated quaternary carbon ( $\delta = 69.0$ ), two methylene ( $\delta = 45.4$  and  $45.3$ ) and a methyl group ( $\delta = 27.3$ ). These observations clearly suggested that compound **12** was an ester of 3-hydroxy-3-methyl glutaric acid (HMG, dicrotaic acid). NMR data found for the acyl moiety were in good accordance with those reported for similar acylated compounds [14, 15]. Moreover, the chirality of the acyl moiety was found to be *S* by comparing the  $^1H$  and  $^{13}C$  NMR data of the compounds having a (*S*)-3-hydroxy-3-methyl glutaric acid moiety [16–18]. The

downfield shifts observed for the C-6'' ( $\delta = 64.1$ ) as well as for the H<sub>2</sub>-6'' ( $\delta = 4.36$  and  $3.93$ ) resonances of the  $\beta$ -D-glucopyranose moiety revealed the similar esterification site as in **11**. The site of esterification was also supported by a long-range correlation between the oxymethylene protons of the glucopyranose moiety (H<sub>2</sub>-6'') and the carbonyl carbon ( $\delta = 170.5$ ) of the HMG moiety in the HMBC experiment. Thus, the structure of **12** was established as the new 6''-*O*-[(*S*)-3-hydroxy-3-methylglutaroyl]-isoorientin.

All other isolated compounds (**1–7**) were identified by comparison of their physical and spectroscopic data with literature values and by comparison with authentic samples. Compound **1** was identified as chlorogenic acid (5-*O*-caffeoyl quinic acid) based on its  $^1H$  NMR spectra measured in D<sub>2</sub>O, DMSO and MeOD [19]. Compound **2** was identified as adenosine [20]. Compounds **3** and **4** were identified as L-tryptophan [21] and L-phenylalanine [22] according to their  $^1H$  and  $^{13}C$  NMR data, respectively. Compounds **5–7** were identified as aloe-emodin [23], aloe-emodin acetate [24], and chrysophanol 8-*O*-gentiobioside [25], respectively. Compounds **5–7** were also isolated from *Kniphofia* species which is also a member of Asphodelaceae like *Asphodelus* [24].

Investigation of the air-dried leaves of *A. aestivus* resulted in the isolation of the same compounds except acylated isoorientin derivatives **11** and **12**, indicating a deacylation during the drying process.

#### Acknowledgements

We are very grateful to Dr. K. Mayer and Mr. J. Kiermaier (both Zentrale Analytik der Naturwissenschaftlichen Fakultät IV der Universität Regensburg) for measuring the ESI and LSI mass spectra.

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